U.S. Environmental Protection Agency
Office of Research and Development
National Exposure Research Laboratory
Exposure Methods and Measurement Division
Environmental Chemistry Branch

Amendment #1 to the QAPP entitled:

Detection, Evaluation, and Assignment of Multiple Poly- and Perfluoroalkyl Substances (PFAS) in Environmental Media from an Industrialized Area of New Jersey (D-EMMD-0031345-QP-1-0)

Effective May 2, 2018

QA Category: A

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Amendment #1 to the Quality Assurance Project Plan (QAPP), for the Athens Laboratory work on soil, sediment and plant samples, entitled:

Detection, Evaluation, and Assignment of Multiple Poly- and Perfluoroalkyl Substances (PFAS) in Environmental Media from an Industrialized Area of New Jersey
(D-EMMD-0031345-QP-1-0, September 14, 2017)

This document is an amendment to the above mentioned QAPP and details revisions to the original QAPP. These revisions are intended to provide clarification to the approach taken to analyze soil, sediment, and plant samples by staff in Athens, GA. This amendment is intended to supplement, not replace, the original document. Nevertheless, if there are differences in detail between the original document and this amendment, this amendment takes precedence. This amendment will be assigned a document control ID of D-EMMD-0031345-QP-1-1.

A7. Quality Objectives and Criteria for Measurement Data

Quality action thresholds: This QAPP prescribes large numbers of statistically based checks of quality, depending on the number of samples and analytes. When numerous statistical tests are performed on a single project, with thresholds defining significant differences based on single tests, the probability of project-level Type I error (i.e., falsely concluding the statistical test indicates failure of compliance with the QAPP objectives) is grossly compounded, a complication long recognized in statistics (Steel and Torrie, 1980) as well as the EPA (Gibbons, 1990). Quality thresholds for action reported in this QAPP reflect this complication. However, as part of the PI's normal practice, depending on specifics of each observation, the project PI will assess quality beyond these criteria even if/when quality action thresholds do not fall out of compliance.

Sample-holding time: Perfluorinated compounds are known to be recalcitrant. Supporting this tenet, in an ongoing experiment, PFOA and $^{13}C_8$-PFOA have been incubated at 25C in the Athens/EPA lab, with samples drawn, extracted and analyzed at selected times to search for evidence of degradation. The most-recent analysis followed 2083 days (5.7 years) of incubation and this sample showed no evidence of degradation (USEPA/Athens Laboratory Notebook 9083, p. 2-3). Based on this, for legacy perfluorinated compounds, sample-holding times will be limited to one year unless specified otherwise in the final data report. For novel compounds, the actual sample-holding time will be identified in the final data report.

Accuracy: For most analytes on this project, certified standards are purchased from Wellington Laboratories (Guelph, ON, Canada). These certified standards are traceable to specific crystalline lots and prepared using NIST and/or NRC traceable external weights using Class A volumetric glassware that is tested against ASTM procedures that are traceable to NIST.

Calibration standards in this project are prepared with mass-unlabeled analytes and mass-labeled matrix internal analytes. The unlabeled and mass-labeled analytes in the prepared calibration standards are from separate lots and will be checked for internal consistence. If these checks

indicate discrepancy of >30% the researcher will prepare new standards for discrepant analyte, describe the discrepancy in the final data report, or will not report that analyte.

For the calibration standards, back prediction of calibration curve points should be within +/- 30% for concentrations >15 pg/g and +/- 5 pg/g for concentrations <15 pg/g with the least-squares calibration line maintaining central tendency.

Precision: For soil, sediment, and plant samples, the objective for replicate-analysis precision is to achieve ±50%. Target precision will be measured for perfluorocarboxylate homologues as the coefficient of variation (COV) between triplicate sample analyses defined as:

$$COV = \frac{s}{\bar{x}}$$

where s is the standard deviation and $\bar{x}$ is the mean

Method sensitivity: Straight solvent-blank samples should be free of analytes to demonstrate control of laboratory contamination. Solids extractions involve numerous blowdown steps with aggressive solvents, and process blanks routinely have low detectable concentrations. These process-blank detections are corrected for statistically as described below in Section B5.

B4. Analytical Methods

Soil, sediment and plant samples will be analyzed for known analytes primarily using a Waters Acquity ultra-performance liquid chromatograph (UPLC) interfaced with a Waters Quattro Premier XE mass spectrometer (MS). Samples also might be analyzed for known analytes using an Agilent 6400 Series triple quad LC/MS system that is not yet delivered to this laboratory.

Research analytical methods on these instruments will be developed specifically for this project with the objective of analyzing compounds identified as potentially relevant through exploratory sample analysis. Quantitation will be performed with stable-isotope-labeled compounds, chosen by the researcher, used as matrix internal standards.

Non-targeted analyses will be performed on samples selected by the researcher using a Waters Acquity UPLC interfaced with a Waters Xevo G2-XS quadrupole time-of-flight (QTof) MS. Using the QTof in MS^e mode, generated molecular features will be sorted by signal strength and the strongest signals plotted in Kendrick mass-defect diagrams calculated using fluorocarbon moiety masses chosen by the researcher as the basis mass defect. These plots will be compared among samples to identify common molecular features that are judged by the researcher to be worthy of further elucidation with software supplied with the instrument and on-line elucidation tools. When these efforts suggest compounds of interest to the project, the QTof can be run again in MS/MS mode with the quadrupole locked on selected precursor masses and the Tof running in full scan to generate mass spectra that will be used to confirm and further elucidate details of the molecular features of interest, and to develop candidate collision transitions to

analyze these compounds on conventional LC/MS (e.g., Quattro Premier XE) using methods described above.

B5. Quality Control

Acetonitrile (ACN) blanks, with no modification, will be run at least every ten analytical runs, but commonly every five or six analytical runs, to allow for inspection of carry-over at the discretion of the researcher. Any carryover identified by the researcher will be addressed by the researcher by rerunning the entire sample series, rerunning portions of the sample series, and/or noting and explaining the observation with the reported data.

Process blanks will be run in numbers chosen by the researcher, but generally on the order of five process blanks per ten field samples (so if samples are extracted in triplicate reps, five process blanks per thirty sample extracts). These process blanks will be used to define sample detection limits as described below. If one or more process blanks confound LOD or LOQ calculations defined below as determined by the researcher, the researcher will correct by rerunning entire sample series, rerunning portions of the sample series, and/or noting and explaining the observation with the reported data.

Check standards will be run in every batch analytical run in numbers chosen by the researcher, but generally at a rate of two check standards per ten field samples. When the project consists of numerous batch runs, the researcher will select standard levels to cover a range of values. The objective for these check standards will be to agree with nominal value, for perfluorocarboxylate homologues within 50%. If one or more check standards fall outside of this range, the researcher will correct by rerunning entire sample series, rerunning portions of the sample series, and/or noting and explaining the observation with the reported data.

Repeated measures will be run in each batch analytical run in numbers chosen by the researcher, but generally at a rate of two per ten field samples. The objective for these repeated measures will be to agree with the primary measure, for perfluorocarboxylate homologues, within 50%. If one or more repeated measures fall outside of this range, the researcher will correct by rerunning entire sample series, rerunning portions of the sample series and/or noting and explaining the observation with the reported data.

Recovery will be calculated for samples using the recovery internal standard $^{13}C_8$-perfluorooctanoic acid (M8C8) which was added to the field samples in known mass before extraction was initiated. The objective for recovery is to fall between 50% and 150% of the nominal value. If recovery falls outside of this range, the researcher will correct by rerunning the entire sample series, rerunning portions of the sample series and/or noting and explaining the observation with the reported data.

For solid samples analyzed at the Athens laboratory, the limit of detection (LOD) and limit of quantification (LOQ) are defined using a two-mean Student's t-test having common, but unknown variance (Steel and Torrie, 1980; Rankin et al., 2016):

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{\sqrt{s^2_{pooled}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}}$$

where t is the test statistic used to define LOD and LOQ, $\overline{x_1}$ is the mean of each soil, $\overline{x_2}$ is the process-blank mean, s^2 pooled is the pooled sample variance, and numbers of observations are given by $n_1 = 3$ soil replicates and n_2 = the number of process-blanks. The pooled sample variance is defined as:

$$s^2_{pooled} = \frac{(n_1 - 1)\Sigma(x_1 - \bar{x}_1)^2 + (n_2 - 1)\Sigma(x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}$$

To define LOQ and LOD, calculated values of t are compared to critical t values ($t_{critical}$) for a one-tailed t-test, abbreviated $t_{\alpha(1),\upsilon}$ where α is the specified significance level, (1) signifies one-tailed, and υ is the degrees of freedom ($\upsilon = n_1 + n_2 - 2$). By using this standard statistical approach, detection limits are minimized, albeit at the cost of having unique limits for each sample arising from the sample-specific standard deviation among the three replicates. Sample values exceeding the LOQ are reported as process-blank corrected, i.e., reported sample concentrations are analytical concentrations minus mean process-blank values.

B6/B7. Instrument/Equipment Calibration, Testing, Inspection, Maintenance

The Athens Quattro Premier LC/MS is maintained by the researcher and Waters technicians. The calibration of the LC/MS response for individual compounds is based on the calibration curve solutions run for each project. For any batch of samples, if check standards fall out of acceptable range (defined above in B5), a new calibration curve can be generated at the discretion of the researcher. All calibrations are carried out with mass-labeled matrix internal standards. In general, at a minimum, a 7-point calibration curve is analyzed. Calibration is carried out with 1/x weighting and resulting correlation coefficient should be > 0.97. For some very long-chain PFCAs (e.g., C16, C18) quadratic calibrations might be necessary. In these cases, effort will be expended to minimize the range of calibration. For linear calibrations, any samples with concentrations of an analyte more than 10% above the top calibration point require appropriate dilution of the primary sample (preserved in the lab) and complete reprocessing and reanalysis of that sample for that compound. For quadratic calibrations, all samples must fall within the range of the calibration standards.

The Athens Xevo LC/MS system is maintained by Waters technicians. During analytical runs, the mass calibration is benchmarked every thirty seconds by shunting flow from the sample to direct infusion of the reference-mass calibrant, leucine enkephalin. In negative ESI-mode the reference mass is 554.262 Da/esu and in positive ESI mode the reference mass is 556.2766 Da/esu. Mass calibration is performed with a buffered Na-formate solution referencing 16 masses ranging roughly from 100 to 1000 Da. Mass calibration generally is performed within a

week of analytical runs. For data expected to be used for manuscripts, reports and/or papers, mass calibration generally is run the same day as analysis or the day before.

B8. Inspection/Acceptance of Supplies and Consumables

For the Athens laboratory, materials will be used that are as free from contamination to the extent feasible; however, the harsh extraction method required for soil, sediment, char, dispersion and plant samples, may result in detectable levels of analyte accumulated during sample processing. This will be monitored through the preparation and analysis of process blanks as described in Section B5. The LOD and LOQ will be determined with pooled analysis of these blanks as described in B5. Sample values are reported as blank corrected.

References:

Gibbons, RD. 1990. A general statistical procedure for ground-water detection monitoring at waste disposal facilities. Ground Water. 28. 2. p. 235-243.

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Steel, RGD, Torrie, JH. 1980. Principles and Procedures of Statistics: a Biometrical Approach. 2nd ed. McGraw-Hill Book Company. New York, NY. 633 pp.